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FIELD TRIAL OF ATTENUATED SALMONELLA TYPHI LIVE ORAL VACCINE TY21A IN LIQUID AND ENTERIC-COATED FORMULATIONS AND EPIDEMIOLOGICAL SURVEY FOR INCIDENCE OF DIARRHEA DUE TO SHIGELLA SPECIES

ANNUAL REPORT

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In October, 1986, a ramdomized, controlled field trial was initiated in Aera Sur Oriente and Area Norte, Santiago, Chile, to compare the relative and absolute efficacy of three doses of Ty21a vaccine given in enteric-coated capsule or liquid formulation. Intensive clinical and bacteriologic surveillance is being maintained. Preliminary data show that the liquid formulation is associated with a significantly lower attack rate for typhoid fever than the enteric-coated capsule formulation. Surveillance for this trial continues.

Epidemiologic and microbiologic surveillance was initiated in three populatins of children in the Santa Julia neighborhood of Area Oriente, Santiago with the broad objective of preparing a field area where the efficiacy of vaccines against Shigella, enterotoxigenic E. coli and other diarrheal pathogens can be evaluated. In the first years of surveillance diarrhea in young children showed a prominent seasonality with approximately twice as many episodes occuring in the warm season. Similarly, isolation of Shigella was maximal during the warm months. An impressive portion of the episodes of diarrhea in Santa Julia children,

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FOREWORD

For the protection of human subjects, the investigators have adhered to policies of applicable federal law 45 CFR 46.

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I. A FIELD TRIAL COMPARING THE EFFICACY OF TWO DIFFERENT FORMULATIONS OF TY21A LIVE ORAL TYPHOID VACCINE

The transmission of typhoid fever is favored by circumstances where food and water vehicles can readily become contaminated. Historically, typhoid fever was a major problem of military personnel in the 19th century, and was rampant, for example, among U.S. troops in Cuba during the Spanish-American War (1). Inactivated vaccines consisting of killed whole Salmonella typhi organisms inoculated parenterally have been used in military personnel since the Boer War in South Africa. Their routine use in the U.S. Army after the First World War resulted in greatly diminished reported incidences of typhoid fever (2). A series of randomized, placebo-controlled field trials carried out in the 1950s and 1960s under the auspices of the World Health Organization established unequivocally that the acetone-inactivated and heat-phenol-inactivated whole cell typhoid vaccines provide significant protection for at least three years (up to seven years in one trial in Guyana) (3-6). Several of the aforementioned vaccines used in these controlled field trials were prepared at the Walter Reed Army Insitute of Research (7). Although moderately protective, the killed whole cell vaccines are not satisfactory immunizing agents for use in adult U.S. military personnel or in children in typhoid-endemic areas because of the frequent and severe adverse reactions that they elicit. In controlled double-blind studies, the killed whole cell parenteral vaccines cause fever in approximately 25% of recipients, incapacitating malaise leading to absenteeism in 15-20% and notable local adverse reactions in about 50% (5,8,9). Consequently, a high priority has been given to identifying a well-tolerated yet protective typhoid vaccine.

Attenuated S. typhi strain Ty21a developed by Germanier and Furer

(10) was proposed by those investigators as a possible live oral vaccine candidate. In preliminary studies in North American volunteers, Ty21a, even in doses as high as 10^{11} viable organisms, was well-tolerated (11). Furthermore, in experimental challenge studies in volunteers Ty21a was highly protective (11).

Based on the encouraging results in North American volunteers, the first field trial of efficacy of Ty21a was carried out in 6-7 year old schoolchildren in Alexandria, Egypt where approximately 16,000 children received three doses of vaccine (on Monday, Wednesday and Friday of one week) and 16,000 received placebo (12). Lyophilized vaccine was reconstituted in the field and children ingested the liquid suspension several minutes after chewing a 1.0 gm $NaHCO_3$ tablet to neutralize gastric acid. After three years of surveillance 22 culture-confirmed cases were recorded in the placebo group, while only one case occurred in the vaccine group (96% vaccine efficacy) (12).

Subsequently a more practical formulation of Ty21a was developed consisting of lyophilized vaccine in enteric-coated capsules. This practical formulation was evaluated in several large-scale field trials in Santiago, Chile supported by the U.S. Army Medical Research and Development Command and the World Health Organization (3,13,14). In the second trial, which was initiated in Area Occidente in August, 1983, the enteric-coated capsule formulation was compared with a commercial formulation consisting of lyophilized vaccine and $NaHCO_3$ in gelatin capsules (14). The enteric-coated capsule formulation was significantly more protective than the gelatin capsule/$NaHCO_3$ formulation. Three doses of Ty21a in enteric-coated capsules given within one week has provided 65% protection that has endured for at least 5 years (Table 1) (14). The 65% vaccine efficacy for at least five years conferred by three doses of

Ty21a in enteric-coated capsules given within one week is quite a credible performance by a vaccine. Nevertheless, it is notably less than the 96% protection conferred by Ty21a in Alexandria, Egypt where a liquid formulation of vaccine was used. There are many other possible explanations to account for the differences in efficacy between the Alexandria and Area Occidente trials, including human genetics affecting immune response to vaccine, antigenic differences between prevalent S. typhi strains, possible distinct modes of transmission that might affect inoculum size and thereby vaccine efficacy, and differences in surveillance methods (active in Alexandria, passive in Occidente). Nonetheless, the difference in formulation of vaccine between the two trials is the one variable that could be directly tested. Accordingly, in October, 1986, a randomized, controlled field trial was initiated in Area Sur Oriente and Area Norte, Santiago, Chile to compare the relative and absolute efficacy of three doses of Ty21a vaccine given in enteric-coated capsule or liquid formulation.

Trial Design

In October and November, 1986, a total of 98,956 pre-randomized schoolchildren, including 63,979 in Area Sur Oriente and 34,977 in Area Norte, received Ty21a vaccine or placebo in either enteric-coated or liquid formulation. A total of 84,836 children, 6-19 years of age, received all three scheduled doses of vaccine or placebo within the eight day immunization period. The Area Norte participants represent children who entered school subsequent to the initiation of the 1982 field trial in Area Norte so 91% are less than 10 years old.

The "liquid" formulation consisted of two sachets, one containing a single dose of 1-3 x 10^9 lyophilized vaccine organisms (or lyophilized lactobacilli serving as the control preparation) and the other containing

a buffer. One sachet of buffer and one of vaccine (or lactobacilli control preparation) was emptied into a disposable cup, 100 ml of water was added, the contents were stirred and the suspension was ingested by the schoolchild.

Because of ethical considerations, the size of the control group was kept to the minimum required to obtain statistically significant measures of vaccine efficacy; only one-eighth of the participating children received the control preparation. In order to maintain blindness, there were eight separate coded liquid preparations, of which seven contained vaccine and one held lactobacilli, the control preparation. Similarly, among the eight coded enteric-coated capsule preparations, seven had vaccine and one contained lactobacilli.

Results of Surveillance

In Area Sur Oriente approximately 90% of all health care visits take place in health centers (consultorios) of the National Health Service, the corresponding percent for Area Norte is 85%. Accordingly, intensive clinical and bacteriologic surveillance was maintained in the consultorios and hospitals of these areas. Two 6 ml blood cultures 30 minutes apart were obtained from all children presenting as outpatients to consultorios or hospitals with a clinical syndrome suspect of being typhoid fever. From hospitalized pediatric inpatients with presumed typhoid fever, three blood cultures were obtained. Only culture-confirmed cases were utilized in computation of incidence rates, data analysis and statistical comparisons.

Surveillance began in late November, 1986. Cumulative results of surveillance which include the period of observation from 11/1/86 to 3/15/89 are summarized in Table 2. The code remains unbroken. Thus it is not possible to calculate absolute vaccine efficacy. Nevertheless,

although the recipients of placebo cannot be identified, it is known who received the liquid and who received the enteric-coated capsule formulations of Ty21a. Consequently, it is possible even at this time to compare the relative efficacy of the two different formulations, recognizing that approximately one-eighth of the recipients of each of these two formulations received lactobacilli control preparation, rather than vaccine. As shown in Table 2, the preliminary data already show that the liquid formulation is associated with a significantly lower attack rate for typhoid fever than the enteric-coated capsule formulation. Surveillance of this trial must continue to build up sufficient numbers of typhoid cases to be able to have a valid measure of the absolute efficacy conferred by Ty21a in each of these formulations.

II. EPIDEMIOLOGICAL STUDIES OF DIARRHEA DUE TO SHIGELLA, ESCHERICHIA COLI AND OTHER AGENTS

Diarrheal diseases represent one of the major sources of morbidity for U.S. military personnel deployed in less-developed areas of the world (15,16). The lack of adequate sanitation and primitive food and personal hygiene practices in the less-developed world and the lack of immunity of young U.S. adults to the prevalent etiologic (mainly bacterial) agents result in high attack rates of traveler's diarrhea among U.S. military personnel. In less-developed areas, incidence rates of diarrheal disease are high among children in the first three years of life (16-19). One consequence of these repeated infections in early childhood is the acquisition of immunity that results in a low incidence of diarrheal disease in indigenous adults and older children (16,20,21). However, when U.S. adults travel to less-developed areas they immunologically resemble infants and young children, not indigenous adults.

Consequently, by studying the epidemiology of diarrheal diseases in young children in less-developed countries, much can be learned that is applicable to the immunoprophylactic control of diarrheal diseases and dysentery among U.S. soldiers. Furthermore, arguably an accurate measure of the ability of candidate anti-diarrheal (e.g. anti-Shigella) vaccines to protect U.S. military under conditions of natural challenge can be derived by assessing the efficacy of such vaccines in protecting indigenous infants and young children.

The Four Arms of the Epidemiological Studies in Santa Julia

Epidemiologic and microbiologic surveillance was initiated in three populations of children in the Santa Julia neighborhood of Area Oriente, Santiago with the broad objective of preparing a field area where the efficacy of vaccines against Shigella, enterotoxigenic E. coli and other diarrheal pathogens can be evaluated in randomized, placebo-controlled double-blind trials. Santa Julia is a densely-populated community of substandard housing (a so-called "poblacion").

In these field studies two separate cohorts, one assembled cross-sectionally by age and the other by admission of newborns, are followed prospectively with active surveillance by twice weekly household visits. In addition, passive surveillance to detect diarrhea due to Shigella, E. coli and rotavirus is maintained at the consultorio and (health center) in Santa Julia and in the Calvo Mackenna Children's Hospital that serves all of Area Oriente.

Thus the various arms of the field studies include:

A. Two Active Surveillance Cohorts:

1. Cross-Sectional Shigella/E. coli Cohort - -

i. The epidemiology of Shigella and diarrheagenic E. coli in cases and controls is recorded in a prospectively followed cross-sectional

cohort of 330 children 0-60 months of age whose households are visited twice weekly by public health nurses.

ii. In a subcohort of 120 of the above 330 children in the case/control study the prevalence of Shigella in the community is monitored by weekly surveillance cultures.

2. Newborn Admission, Comprehensive Etiology Cohort

i. A second cohort of 130 children has been assembled who were admitted to the study as newborns and followed prospectively thereafter with active household surveillance. In these children an extensive survey of the etiology of diarrheal disease (including bacterial, viral and protozoal agents) is being pursued as they grow through infancy and toddler age to reach preschool years.

B. Passive Surveillance Sites --

Passive surveillance is being maintained at the secondary and tertiary health care facilities that serve the Santa Julia community. This arm of the study monitors the frequency of isolation of Shigella and diarrheagenic E. coli among Santa Julia children age 0-48 months who visit the Santa Julia consultorio or who are admitted to the Calvo Mackenna Children's Hospital with diarrheal disease.

THE EPIDEMIOLOGY OF SHIGELLA INFECTIONS IN SANTA JULIA

Field Methods and Definition of Diarrhea

Of the 360 children initially enrolled into the cross-sectional cohort arm of the study in November, 1986, some of have been lost to follow-up by out-migration from Santa Julia, while others upon reaching 60 months of age were "graduated" from the cohort. Public health field nurses working on the project visit the houses of participating children twice weekly to detect cases of diarrheal disease. Diarrhea is defined

as an overt change in the normal stool pattern of the child characterized by an increase in the frequency and a decrease in the consistency of stools to an unformed state noted by the child's caretaker; this must comprise passage of at least three loose stools in a 24 hour period. If a child has diarrhea, a pre-determined, matched control child in the cohort is visited to ascertain that the control is without diarrhea. A stool specimen or rectal swab is obtained on two consecutive days from the child with diarrhea and from his/her age-matched control and examined for Shigella and the different categories of diarrheagenic E. coli.

Laboratory Methods for Shigella

Stool specimens are transported to the laboratory in tubes containing buffered glycerol saline transport medium (the preferred transport for Shigella) (22) and are immediately plated onto MacConkey's, Salmonella-Shigella, and xylose-lysine-desoxycholate agar. Lactose-negative colonies are picked to identify Shigella which are subcultured to triple sugar iron slants and incubated overnight. Suspicious isolates are confirmed by further standard biochemical and serological tests.

Shigella Infections in the Cross-Sectional Cohort Study

Table 3 summarizes by month the number of children under surveillance in the cross-sectional cohort, the episodes of diarrhea that occurred, the number of matched healthy controls obtained, the total number of coprocultures gotten from each group, and the isolation of Shigella from cases and controls. Evidence of the diligence and tenacity of the study nurses working in the field is demonstrated by the fact that a matched control child was successfully cultured for 903 of the 951 episodes of diarrhea (95%) that occurred during these two years of surveillance. During these first two years of surveillance, diarrhea in

the Santa Julia children showed a prominent seasonality with almost twice as many episodes (602) occurring in the warm season from November through April as in the cool half of the year (349). Similarly, the isolation of Shigella from cases was greater during the warm months (78 isolations) than during the cool half of the year (18 isolations) ($p<0.0001$). The difference in isolation rate of Shigella from cases (10.1%) versus controls (3.1%) was highly significant ($p<0.00001$).

Table 4 analyzes the occurrence of Shigella infections by age group. Displayed are the child months of observation by age group, the number of episodes of diarrhea, the mean episodes per 12 child months of observation, and the occurrence of all Shigella for the period from November 1, 1986 until October 31, 1988. The rate of diarrheal illness, expressed as the incidence per 12 child months of observation, is highest in the first year of life and steadily decreases thereafter. The incidence of Shigella infection is lowest in the two age extremes of the cohort, i.e. in infants < 12 months of age and in the oldest preschool children ($\geq$ 48 months of age). The peak incidence of Shigella infection occurred in children 36-47 months of age where the rate was 0.2 Shigella infections/12 child months of observation.

Weekly Prevalence of Shigella Carriage in A Subcohort

In order to quantify the magnitude of the reservoir of Shigella, in particular to determine the prevalence of subclinical infections, a subcohort of 158 children of all ages was randomly selected from among the cross-sectional cohort of 330 prospectively followed children. These 158 children had weekly stool cultures collected to detect Shigella. Forty-nine percent of the 158 children had positive cultures for Shigella. While most infections were documented during the warm months of the year (December through March), subclinical infections were also

recorded in the cool months, suggesting that in this way Shigella may carry over to the next warm season when conditions are again favorable for enhanced transmission. Many asymptomatically-infected children shed Shigella for several weeks consecutively.

Health Center and Hospital-Based Passive Surveillance for Shigella

In addition to active prospective surveillance of a cohort of 330 children from 0-60 months of age involving twice weekly household visits to study the epidemiology of Shigella infection, passive surveillance is being maintained at the Consultorio Santa Julia and at the Calvo MacKenna Children's Hospital (which serves Area Oriente). At the consultorio this involves obtaining stool cultures from all children with diarrhea less than 48 months of age who present to the consultorio to detect Shigella and diarrheagenic E. coli.

At the hospital, the records of children < 5 years of age admitted with acute diarrheal disease are reviewed daily (Monday to Friday) to identify children from Santa Julia. These children are cultured to detect Shigella and diarrheagenic E. coli. Both in the hospital and at the consultorio any children from the cohort of 330 are identified to record those episodes of diarrhea that were sufficiently severe to require the parent to take the child to a health care facility or to require hospitalization.

The isolation of Shigella by month from children with diarrhea seen at the consultorio and in the hospital is summarized in Table 5. Virtually an identical pattern of seasonality of isolation of Shigella was found in the consultorio as in active surveillance of the cross-sectional cohort study (Table 3).

Severity of Illness due to S. sonnei vs S. flexneri

The relative frequency of occurrence of Shigella by species is shown in Table 6, comparing the active surveillance cohort, health center and hospital isolations. The relative proportion of isolations of S. sonnei and S. flexneri were almost identical in the active surveillance cross-sectional cohort and in the passive surveillance consultorio sample; 42-44% were S. sonnei and 52% were S. flexneri (the small remainder were S. boydii). However, in the hospital surveillance sample only 28% of the isolations were S. sonnei, while 72% were S. flexneri (Table 6). Compared to the combined experience of the cross-sectional cohort and the consultorio, the proportion of S. flexneri isolates was significantly higher among hospitalized cases (23/32, 71.9% vs 114/220, 51.8%, p=0.05). This is consistent with the view of epidemiologists and infectious disease consultants that shigellosis due to S. flexneri is generally more severe than that due to S. sonnei and thus may more readily lead to hospitalization.

Rate of Isolation of Shigella in Relation to Surveillance Method (Active vs Passive) and Sampling Site

It has been a common supposition among epidemiologists that Shigella are isolated from a somewhat greater proportion of children with diarrhea seen in hospitals and health centers than with diarrhea detected by active household surveillance because the first two represent a more severe spectrum of clinical illness and Shigella is more prone to cause diarrheal illness of greater severity. Heretofore, it was not readily possible to critically test this hypothesis from other published studies undertaken elsewhere. Constraints of earlier studies include: 1) the combination of active and passive surveillance of the same base population was not simultaneously maintained at the household, health center and hospital levels and reported as such; 2) different

bacteriologic techniques were utilized in hospital versus field surveillance; 3) the populations were not of similar age structure for comparison.

In the Santa Julia study we were able to overcome these limitations in a deliberate attempt to address the question. In Table 7, the relative frequency of isolation of Shigella from coprocultures of children with diarrhea, by age group, is compared for children from the active surveillance cohort, from the consultorio and from the hospital. Indeed, some significant differences in rate of isolation were found. For example, among children in the first year of life with diarrheal illness, Shigella was significantly more often isolated from children of that age admitted to hospital with diarrheal disease than among those with diarrhea detected by household visits or at the consultorio. Similarly, among toddlers (i.e 12-23 month olds) admitted to hospital with diarrheal disease, Shigella was more often isolated (4 of 14, 28.6%) than it was among toddlers with diarrhea detected by household surveillance (11 of 157, 7.0%) ($p = 0.023$, Fisher's exact test, 2 tails).

Isolation of Shigella in Relation to Clinical Syndrome

In Table 8 the isolation of Shigella is analyzed in relation to the clinical form of diarrheal disease. In the presence of overt dysentery, characterized by the presence of blood and mucus in diarrheal stools, Shigella was isolated significantly more often than from children who had simple diarrhea without blood and mucus.

THE EPIDEMIOLOGY OF E. COLI DIARRHEA IN SANTA JULIA

Active Surveillance of a Cross-Sectional Cohort for and E. coli Diarrhea

This prospective cross-sectional cohort study was also meant, as

rapidly as possible, to explore Santa Julia as a possible site to evaluate candidate vaccines against enterotoxigenic E. coli and other diarrheagenic E. coli in controlled field trials.

Laboratory Methods for Identifying Diarrheagenic E. coli

From the same stool specimens that are cultured for Shigella, bacteriological methods are applied to identify diarrheagenic E. coli. From the MacConkey's agar plates lactose-negative colonies are picked to identify enteroinvasive Escherichia coli by means of a DNA probe (23). Lactose-positive colonies are examined to detect enterotoxigenic E. coli (ETEC) (24), enteroinvasive E. coli (EIEC) (23), enteropathogenic E. coli (EAF) (25), and enterohemorrhagic E. coli (EHEC) (26) by DNA probe technique. E. coli strains that adhere to HEp-2 cells in a diffuse pattern of adherence (24) are also detected by DNA probe methodology.

During the first two years of this research contract, a considerable effort has been expended to introduce from the Center for Vaccine Development into the Microbiology Unit of the University of Chile, DNA probe technology that employs a colorimetric (biotin-labelled) rather than a radioisotopic signal. The probe for heat-stable enterotoxin (ST) comes from a commercial kit consisting of a synthetic oligonucleotide conjugated linked to alkaline phosphatase as the signal. All the other probes represent specific cloned fragments of DNA that are purified, made single stranded and biotin-labelled by the investigators for use as diagnostic reagents.

Table 9 shows the identification of diarrheagenic E. coli by probe technique from cases of diarrhea and controls tested during the period from November 1, 1986 to October 31, 1988. In this cross-sectional cohort, ETEC were the most common diarrheagenic E. coli identified by DNA probe; EPEC were the next most common category of diarrheagenic E. coli.

EIEC and EHEC were quite uncommon. The isolation of the various categories of diarrheagenic E. coli in relation to age is summarized in Table 10.

As shown in Table 9, ETEC were isolated significantly more often from cases (97/832) than from controls (56/824) ($p<0.00001$). It is interesting to note that several previous studies of diarrheal disease in Latin America have failed to show a significant difference in rate of isolation of ETEC from cases as compared with controls (28,29).

Table 9 shows the isolation of ETEC by month. It is apparent that ETEC diarrheal infections peak in the warm months of the year. In the warm months of November through April, 79 cases of ETEC-associated diarrhea were observed over the two years of surveillance. In contrast, in these two years, during the cool months of May through October only 45 cases of ETEC-associated diarrhea were observed.

The toxin phenotype of the ETEC infections is shown in Table 11. Of the 97 cases of ETEC-associated diarrhea, 17 (18%) were due to LT/ST strains, 54 (56%) to LT-only strains and 26 (27%) to ST-only strains; 3 cases grew yielded both LT-only and ST-only isolates. The occurrence of ETEC infections by age and by toxin phenotype is shown in Table 12.

Serotyping of a representative sample of the ETEC strains is being carried out through the kind assistance of Hermy Lior, Chief of the E. coli Reference Laboratory of the Canadian Communicable Disease Center, Ottawa. During the next year we will also be systematically identifying the fimbrial colonization factor antigens associated with the ETEC strains isolated from Santa Julia children.

In the cross-sectional cohort arm of the study there was no difference in the rate of isolation of EPEC from cases and controls. Levine and Edelman have reported that case/control studies generally only

show a significant difference in isolation rates of EPEC when children less than six months of age are examined. As shown in Table 10, this arm of the Santa Julia study includes only a small experience of surveillance of infants less than six months of age; thus it is not surprising to observe no significant differences for EPEC. In contrast, as will be shown below, in the newborn cohort study, which detected more episodes of diarrhea in very young infants followed from birth, there was a significant difference in isolation rate of EPEC between cases and controls.

There exists considerable debate over whether E. coli that exhibit the diffuse pattern of adherence in the HEp-2 cell assay cause diarrhea. In some field studies such strains have been isolated significantly more often from cases than from controls (29). In other studies they have been found with equal frequency in both cases and controls (27,37,39). Moseley and coworkers (38) developed a DNA probe to identify E. coli that exhibit diffuse adherence (DA-E. coli) and Levine et al (27) showed the probe to be moderately sensitive and highly specific. The use of this probe in the cross-sectional cohort study is summarized in Table 9. DA-E. coli exhibited that same seasonal pattern as Shigella and ETEC, being notably more common in the warm season. Over two years of surveillance DA-E. coli were found associated with 18.1% of cases of diarrhea and 14.0% of controls. Because of the large sample sizes, this difference is statistically significant (p=0.024). However, the biological significance of this difference is less clear in view of the low relative risk (1.3).

The isolation of the different categories of diarrheagenic E. coli as detected by DNA probes from cultures of children with diarrhea seen at the consultorio or the hospital is shown in Table 13.

NEWBORN PROSPECTIVE COHORT STUDY OF THE ETIOLOGY OF DIARRHEA

Commencing in March, 1987, 12 newborn infants each month were entered into the infant cohort study. The enrollment of infants into this study for the period up to October 31, 1987 is shown in Table 7; 52% are male and 44% are first-borns. Table 14 also shows the occurrence of diarrhea in this cohort by calendar month and in relation to the age of the children at the time of onset of diarrhea. Also shown are the number of days of diarrheal disease morbidity in the cohort, the mean duration of diarrhea and the percentage of cases in which the duration of diarrhea exceeded 14 days. For each infant with diarrhea, an age-matched healthy control infant is selected to be cultured as well. The stool cultures from this arm of the study have not yet been completely tested for diarrheagenic E. coli (tests with the probe for ST have not been completed). Nevertheless, the specimens have been submitted to routine coproculture, culture for Campylobacter, detection of rotavirus by ELISA (30) and microscopy for protozoal pathogens. These results for the infants with diarrhea, by age group, are shown in Table 15.

Prospective surveillance of infants from the perinatal period is a painstaking but highly sensitive method to detect pathogen-specific differences in attack rate between children with diarrhea and healthy controls. In this type of study one has the cumulative experience of each child as well as the comparison with a control. Thus, it is not surprising that using this epidemiological methodology a significant difference was detected between cases and controls for EPEC (EAF-positive E. coli), while such a difference was not detected in the cross-sectional cohort. Among children with diarrhea, EPEC were isolated at the rate of 22 cases/1913 child months of observation versus 9 cases/1913 child months of observation in the controls (p=0.029). EPEC diarrhea peaked

in the 4-6 and 7-9 month old age groups.

The most striking pathogen was rotavirus; 32 cases were recorded during the 1913 child months of observation of the cohort versus 3 cases in the controls ($p<0.000001$). Other pathogens that were found significantly more frequently in cases of diarrhea than in controls are Shigella ($p=0.039$) and Yersinia enterocolitica ($p=0.0063$).

Comment

The mean number of episodes of diarrhea per child per year in the Santa Julia cohort admitted as newborns and followed prospectively thereafter is notably less than has been recorded for rural village children in Bangladesh prospectively surveyed for diarrheal disease (17) or for young children in rural Northeastern Brazil (the least-developed part of that country) (18). Nevertheless, an impressive portion of the episodes of diarrhea in Santa Julia children, particularly during summer, are due to Shigella and E. coli pathogens. These data corroborate the seroepidemiologic studies of Levine et al (21) reported several years ago which showed that among Santiago children 3-5 years of age the prevalence and mean titer of LT antitoxin (a measure of past infection with LT-producing ETEC) was as high as that found in Bangladeshi children of the same age. During the summer months in Santa Julia it is likely that Shigella is readily transmitted from child to child by direct contact involving small inocula. Such transmission, which is dependent on personal hygiene practices, is apparently little affected by the widespread availability of potable water in the poblacion. The weekly Shigella prevalence study of a subcohort of 120 children of different ages has documented that throughout the year a rather considerable reservoir of Shigella infection exists in the pediatric population of Santa Julia. When warm season arrives, it is from this sizable reservoir

that enhanced transmission of Shigella to susceptibles begins.

ETEC and EPEC, also commonly associated with diarrhea in summer, are more likely transmitted by contaminated foods. Most households lack refrigerators for food preservation in summer. The high incidence rates of Shigella, ETEC and EPEC infection make Santa Julia a suitable place for testing the efficacy of vaccines against these agents.

Laboratory studies have only just begun to determine the frequency of isolation of entero-aggregative E. coli (EAggEC), a recently-described putative new category of diarrheagenic E. coli (27,31,32). Two DNA probes have recently been developed at the CVD which appear to be approximately 90% sensitive in identifying EA-AggEC strains from Chile and are highly specific. It is anticipated that when probes of this type are properly standardized they will be transferred to the laboratory in Chile.

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Table 1.

Evaluation of the efficacy of three doses of the enteric-coated capsule formulation of Ty21a live oral vaccine given within one week in Area Occidente, Santiago, Chile. Comparison of the efficacy during years 1-3 versus years 4 and 5

	Vaccine* (22,170)	Placebo* (21,906)
Years 1 - 3 (9/83-8/86)		
Cases	23[a]	68[b]
Incidence/10^5	103.7	310.4
Efficacy	66.6%	-
95% confidence interval	47-79	-
Years 4 and 5 (9/86-8/88)		
Cases	12[c]	32[d]
Incidence/10^5	54.1	146.1
Efficacy	63.0%	-
95% confidence interval	29-81	-

* 3 doses, 1-2 days between doses

a vs b, p = 0.0000027, Chi square

c vs d, p = 0.0037

Table 2. Preliminary results for the period of November, 1986 through February, 1989 of a field trial in Area Sur Oriente and Area Norte assessing the efficacy of Ty21a vaccine in liquid or enteric-coated capsule formulations, in comparison with placebo.

Letter codes of Liquid Preparations	No. Children	No. Cases	Incidence per 10^5
B,H,I,J,L,O,P & Q	43,712	29	66.3[a]
Letter codes of Enteric-coated capsule Preparations			
P,G,K,M,N,R,S & T	41,124	60	145.9[b]

a vs b, $p < 0.0001$

Note -- one letter code of liquid and one of enteric-coated capsule formulation, i.e. one-eighth of each group, represent placebo recipients.

Table 3. Isolation of Shigella from cases of diarrheal disease and from age-matched controls. Cohort study of 360 children < 5 years of age, Santa Julia, Santiago, November 1, 1986 to October 31, 1988

Month	No.of Children in the Cohort	Cases of Diarrhea	Diarrhea Cases due to Shigella	Matched Healthy Controls	Shigella in Health Controls
Surveillance Year 1, 11/86-10/87					
1986					
November	359	58	9 (15.5%)	56	0 (0%)
December	356	53	12 (22.6%)	53	3 (5.7%)
1987					
January	353	53	15 (28.3%)	53	2 (3.8%)
February	347	40	6 (15.0%)	40	3 (7.5%)
March	346	60	6 (11.7%)	57	2 (3.5%)
April	342	56	3 (5.4%)	53	0 (0%)
May	339	37	2 (5.4%)	34	0 (0%)
June	337	22	0 (0%)	21	1 (4.8%)
July	335	29	2 (6.9%)	24	1 (4.2%)
August	335	28	2 (7.1%)	27	1 (3.7%)
September	331	26	1 (3.8%)	23	0 (0%)
October	331	26	3 (11.5%)	26	0 (0%)
Surveillance Year 2, 11/87-10/88					
November	331	39	1 (2.6%)	33	1 (3.0%)
December	330	52	3 (5.8%)	49	1 (2.0%)
1988					
January	329	47	1 (2.1%)	45	2 (4.4%)
February	328	45	10 (22.2%)	42	3 (7.1%)
March	323	54	7 (13.0%)	51	2 (3.9%)
April	317	48	3 (6.3%)	47	3 (6.4%)
May	313	33	3 (9.1%)	30	2 (6.7%)
June	313	28	1 (3.6%)	24	0 (0%)
July*	311	18	1 (5.6%)	17	0 (0%)
August**	310	29	0 (0%)	28	0 (0%)
September**	307	29	1 (3.4%)	28	1 (3.6%)
October**	306	42	2 (4.6%)	42	0 (0%)
Total		953	94 (9.9%)[a]	903	28 (3.1%)[b]

* At this time children 5 years of age and older were withdrawn from the cohort and replaced by newborns.
** Children reaching 5 years of age were replaced by newborns.

a vs b, $p < 0.00001$

Table 4. Isolation of Shigella species from diarrhea cases and controls by age. Cohort of 330 children under prospective surveillance, November 1, 1986 to October 31, 1988.

Age Group (months)	Clinical Status	Diarrheal Episodes	Child months of Observation	S. sonnei	S. flexneri	S. boydii	S. dysenteriae	Diarrhea/ 12 Child Months of Observation	Shigella/ 12 Child Months of Observation
0-11	Diarrhea	106	640	4	0	1	0	1.98	0.09
	Controls	-	-	2	0	0	0	-	-
12-23	Diarrhea	240	1470	11	5	0	0	1.96	0.13
	Controls	-	-	1	1	0	0	-	-
24-35	Diarrhea	259	2214	11	14	3	1	1.40	0.16
	Controls	-	-	4	1	3	0	-	-
36-47	Diarrhea	214	1788	10	19	0	0	1.44	0.19
	Controls	-	-	2	8	1	0	-	-
≥ 48	Diarrhea	134	1824	3	11	1	0	0.88	0.10
	Controls	-	-	0	5	0	0	-	-
Totals	Diarrhea	953	7936	39	49	5	1	1.44	0.14
	Controls	-	-	9	15	4	0	-	-

Table 5. Seasonality of isolation of *Shigella* from passive surveillance of children with diarrheal illness cultured at the Santa Julia Consultorio or at the Calvo MacKenna Children's Hospital, 11/1/86 to 10/31/88

	Consultorio				Hospital			
Month	No. Cases Cultured	Total *Shigella* *	*S. sonnei*	*S. flexneri*	No. Cases Cultured	Total *Shigella*	*S. sonnei*	*S. flexneri*
Surveillance Year 1, 11/86-10/87								
1986								
November	54	3 (5.6)+	3	0	11	2 (18.2)	1	1
December	103	21 (20.4)	15	5	22	6 (27.3)	3	3
1987								
January	127	16 (12.6)	15	0	18	6 (33.3)	5	1
February	101	10 (9.9)	8	2	14	0 (0)	0	0
March	117	10 (8.4)	4	5	13	1 (7.7)	0	1
April	77	8 (10.4)	3	5	10	1 (10)	0	1
May	49	6 (12.2)	4	1	7	1 (14.3)	0	1
June	43	1 (2.3)	0	0	2	0 (0)	0	0
July	31	3 (9.7)	0	3	7	0 (0)	0	0
August	26	3 (11.5)	1	2	4	0 (0)	0	0
September	35	2 (5.7)	0	1	3	0 (0)	0	0
October	31	2 (6.5)	0	2	9	0 (0)	0	0
Surveillance Year 2, 11/87-10/88								
November	51	1 (2.0)	0	1	14	3 (21.4)	0	3
December	64	6 (9.4)	0	6	16	1 (6.3)	0	1
1988								
January	76	11 (14.5)	2	8	18	3 (16.7)	0	3
February	92	13 (14.1)	1	12	12	3 (25.0)	0	3
March	75	3 (4.0)	0	3	11	1 (9.1)	0	1
April	35	4 (11.4)	2	2	11	3 (27.3)	0	3
May	12	2 (16.7)	1	1	5	1 (20.0)	0	1
June	15	0	0	0	7	0 (0)	0	0
July	13	1 (7.7)	0	1	1	0 (0)	0	0
August	15	0	0	0	2	0 (0)	0	0
September	39	2 (5.1)	1	1	3	1 (33.3)	0	1
October	30	2 (6.7)	0	2	9	0 (0)	0	0
Total	1311	130 (9.9)	60	64	229	33 (14.4)	9	24

* The few isolates that were not *S. sonnei* or *S. flexneri* were *S. boydii*.
+ (Percent of cases due to *Shigella*).

Table 6. Relative importance of Shigella species among Shigella strains isolated in the active surveillance cohort, at the consultorio or at the hospital, November 1, 1986 to October 31, 1988

Sampling Site	Total Shigella Isolates	No. (%) sonnei	No. (%) flexneri	No. (%) boydii
Active surveillance cohort	94	39 (41.5)	49 (52.1)[a]	5 (5.3)
Passive surveillance:				
Consultorio	130	60 (46.2)	64 (49.2)[b]	6 (4.6)
Hospital	33	9 (27.3)	24 (72.7)[c]	0 (0)
Total	257	108 (42.0)	137 (53.3)	11 (4.3)

* One case due to S. dysenteriae.

Chi square: a + b vs c, p= 0.05

Table 7. A comparison of the relative frequency of isolation of Shigella by sampling site and by age group, Santa Julia, November 1, 1986 to October 31, 1988

Sampling site	Age Group (mos.)	No. Diarrheal Episodes Cultured *	No. Episodes (%) due to Shigella
Active surveillance cohort	0 - 11	106	3 (2.8)
	12 - 23	240	14 (5.8)
	24 - 35	259	22 (8.5)
	36 - 47	214	27 (12.1)
	≥ 48	134	13 (9.7)
Passive surveillance			
Consultorio	0 - 11	487	25 (5.1)
	12 - 23	468	61 (13.3)
	24 - 35	171	23 (13.5)
	36 - 47	94	12 (12.8)
	≥ 48	99	9 (9.1)
Hospital	0 - 11	177	16 (9.0)
	12 - 23	34	9 (26.5)
	24 - 35	13	6 (46.2)
	36 - 47	3	0 (0.0)
	≥ 48	2	2 (100.0)

* Only one culture per child per episode was obtained for children seen at the consultorio and at the hospital. Therefore, for purposes of comparison, only the first culture from the active surveillance cohort was included in the analysis.

Table 8. A comparison of the rate of isolation of Shigella from cases of diarrhea versus cases of dysentery*

Site of Sampling	Diarrhea Total Cases	Diarrhea No. (%) with Shigella	Dysentery Total Cases	Dysentery No. (%) with Shigella	
Active surveillance cohort	953	94 (9.9)	72	25 (34.7)	$p < 0.000001$
Consultorio	1311	130 (9.9)	127	38 (29.9)	$p < 0.000001$
Hospital	229	33 (14.4)	51	20 (39.2)	$p < 0.00022$
Total	2493	257 (10.3)	250	83 (33.2)	$p < 0.000001$

* Dysentery is defined as the presence of frank blood and mucus in the diarrheal stool.

Table 9. Isolation of diarrheagenic E. coli by DNA probe methodology in cases of diarrhea and controls in a cohort of 330 children < 5 years of age followed prospectively by active surveillance involving twice weekly household visits.
November 1, 1986 to October 31, 1988.

			Results with DNA Probes:*									
			ETEC		EAF		EIEC		EHEC		DA	
Month	Cases	Controls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls
Surveillance Year 1, 11/86-10/87												
1986												
November	58 (7)+	56 (8)+	0	0	0	1	0	0	0	0	1	0
December	53 (53)	53 (51)	9	3	1	0	0	1	1	0	1	4
1987												
January	53 (53)	53 (51)	9	5	1	2	1	2	0	1	14	9
February	40 (39)	40 (38)	6	3	2	0	1	3	0	0	10	4
March	60 (59)	57 (56)	14	6	6	2	1	0	1	1	9	10
April	56 (51)	53 (53)	7	9	1	1	2	2	0	0	12	11
May	37 (34)	34 (34)	1	0	2	2	3	0	0	1	14	7
June	22 (20)	21 (20)	1	0	0	1	1	0	0	1	7	7
July	29 (24)	24 (23)	1	0	0	1	1	0	1	0	9	8
August	28 (24)	27 (24)	1	2	1	1	1	2	0	1	7	8
September	27 (21)	23 (21)	0	1	0	0	0	0	3	1	6	3
October	26 (23)	26 (24)	1	1	0	0	1	1	0	0	5	1
Surveillance Year 2, 11/87-10/88												
November	39 (32)	33 (32)	1	0	0	0	1	0	0	0	1	2
December	52 (49)	49 (49)	9	8	0	0	0	0	0	1	3	7
1988												
January	47 (42)	45 (44)	8	4	1	3	4	1	0	0	13	4
February	45 (42)	42 (42)	4	2	4	1	1	1	0	0	10	7
March	54 (51)	51 (50)	11	3	1	2	1	2	0	1	10	7
April	48 (46)	47 (47)	1	2	1	2	4	1	0	0	7	8
May	33 (29)	30 (29)	3	1	2	2	1	1	0	0	7	7
June	28 (24)	24 (23)	2	2	0	1	0	0	0	0	5	1
July	18 (17)	17 (17)	1		0	0	0	0	0	2	0	0
August	29 (27)	28 (27)	2	1	1	2	1	1	0	1	0	0
September	29 (26)	28 (24)	1	0	0	0	0	0	1	4	0	0
October	42 (39)	42 (37)	4	2	2	0	0	0	1	0	0	0
Total	953 (832)	903 (824)	97[a]	56[b]	26	24	25	18	8	15	151[c]	115[d]

* Only lactose-positive strains have been tested so far

\+ (Number of episodes where cultures were tested with DNA probes)

Note -- ETEC = enterotoxigenic E. coli; EPEC = enteropathogenic E. coli; EIEC = enteroinvasive E. coli; EHEC enterohemorrhagic E. coli; DA = E. coli that manifest the diffuse pattern of adherence to HEp-2 cells.

a vs b, p =0.00086, chi square

c vs d, p = 0.024

Table 10. Isolation of diarrheagenic E. coli in cases and controls by age group in a cohort of 330 children under prospective household surveillance from 11/1/86 to 10/31/88

Category of E. coli	Age Group (mos.)	Cases	Controls
ETEC *			
	0 - 11	13/90 (14.4%)**	3/89 (3.4%)
	12 - 23	22/218 (10.1%)	19/214 (8.9%)
	24 - 35	29/225 (12.9%)	11/225 (4.9%)
	36 - 47	22/180 (12.2%)	13/176 (7.4%)
	≥ 48	11/119 (9.2%)	10/120 (8.3%)
EPEC			
	0 - 11	8/90 (8.9%)	3/89 (3.4%)
	12 - 23	8/218 (3.7%)	8/214 (3.7%)
	24 - 35	6/225 (2.7%)	9/225 (4.0%)
	36 - 47	2/180 (1.1%)	4/176 (2.3%)
	≥ 48	2/119 (1.7%)	0/120 (0%)
EIEC			
	0 - 11	0/90 (0%)	2/89 (2.2%)
	12 - 23	7/218 (3.2%)	7/214 (3.3%)
	24 - 35	5/225 (2.2%)	4/225 (1.8%)
	36 - 47	7/180 (3.9%)	3/176 (1.7%)
	≥ 48	6/119 (5.0%)	2/120 (1.7%)
EHEC			
	0 - 11	1/90 (1.1%)	2/89 (2.2%)
	12 - 23	3/218 (1.4%)	5/214 (2.3%)
	24 - 35	2/225 (0.9%)	2/225 (0.9%)
	36 - 47	1/180 (0.6%)	2/176 (1.1%)
	≥ 48	1/119 (0.8%)	4/120 (3.3%)

* All ETEC infections of any genotype (i.e. LT/ST, LT or ST.)

** No. positive/No. of episodes from which E. coli were tested by DNA probes.

Table 11. Isolation of enterotoxigenic E. coli by DNA probe methodology in cases of diarrhea and controls in a cohor of 360 children < 5 years of age followed prospectively by active surveillance involving twice weekly household visits November 1, 1986 to October 31, 1988.

			Results with DNA Probes:									
			LT/ST		ST only		LT only		ST+LT only		ALL ETEC	
Month	Cases	Controls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls	Cases	Ctrls
Surveillance Year 1, 11/86-10/87												
1986												
November	58 (7)+	56 (8)+	0	0	0	0	0	0	0	0	0	0
December	53 (53)	53 (51)	4	0	2	0	3	3	0	0	9	3
1987												
January	53 (53)	53 (51)	2	0	3	2	5	3	0	0	9[a]	5
February	40 (39)	40 (38)	0	0	0	0	6	3	0	1	6	3
March	60 (59)	57 (56)	3	0	2	1	7	5	2	0	14	6
April	56 (51)	53 (53)	1	2	1	2	5	6	0	0	7	9[b]
May	37 (34)	34 (34)	0	0	0	0	1	0	0	0	1	0
June	22 (20)	21 (20)	0	0	0	0	1	0	0	0	1	0
July	29 (24)	24 (23)	0	0	0	0	1	0	0	0	1	0
August	28 (24)	27 (24)	0	1	0	1	1	0	0	0	1	2
September	27 (21)	23 (21)	0	0	0	0	0	1	0	0	0	1
October	26 (23)	26 (24)	0	0	0	0	1	1	0	0	1	1
Surveillance Year 2, 11/87-10/88												
November	39 (32)	33 (32)	1	0	0	0	0	0	0	0	1	0
December	52 (49)	49 (49)	3	1	1	2	6	6	0	0	9[c]	8[d]
1988												
January	47 (42)	45 (44)	1	1[c]	4	2	3	2	0	0	8	4[e]
February	45 (42)	42 (42)	1	1[d]	2	1	1	1	0	0	4	2[f]
March	54 (51)	51 (50)	1	1	6	3	4	1	1	0	11[g]	3[h]
April	48 (46)	47 (47)	0	0	0	0	1	2	0	0	1	2
May	33 (29)	30 (29)	0	0	3	1	0	0	0	0	3	1
June	28 (24)	24 (23)	0	0	0	0	2	2	0	0	2	2
July	18 (17)	17 (17)	0	0	0	0	1	1	0	0	1	1
August	29 (27)	28 (27)	0	0	1	0	1	1	0	0	2	1
September	29 (26)	28 (24)	0	0	0	0	1	0	0	0	1	0
October	42 (39)	42 (37)	0	0	1	0	3	2	0	0	4	2
Total	953(832)	903(824)	17[i]	7[j]	26	15	54	40	3	1	97	56

‡ (Number of episodes where cultures were tested with DNA probes)

Note -- LT/ST = Episodes where the E. coli strains have genes for both toxins; ST only = strains have only ST genes; LT only = strains with only LT genes; ST + LT only = cultures where both of these type of strains were found.

a= one culture yielded LT/ST and the other ST-only isolates
b= one culture yielded LT-only and the other ST-only isolates
c= one culture yielded LT/ST and the other ST-only isolates.
d= one culture yielded LT/ST and the other LT-only isolates.
e= one culture yielded LT/ST and the other LT-only isolates
f= one culture yielded LT/ST and the other LT-only isolates.
g= one culture yielded LT/ST and the other ST-only isolates.
h= one culture yielded LT/ST and the other ST-only isolates.

i vs j, p = 0.06, 2-tailed Fisher's Exact test

Table 12. Isolation of enterotoxigenic E. coli in cases and controls by age group. Cohort of 330 children under prospective household surveillance from 11/1/86 to 10/31/88

Category of E. coli	Age Group (mos.)	Cases	Controls
All ETEC			
	0 - 11	13/90	3/89
	12 - 23	22/218	19/214
	24 - 35	29/225	11/225
	36 - 47	22/180	13/176
	> 48	11/119	10/120
	TOTAL	97/832	56/824
LT/ST strains			
	0 - 11	1/90	0/89
	12 - 23	5/218	2/214
	24 - 35	9/225	2/225
	36 - 47	1/180	2/176
	> 48	1/119	1/120
	TOTAL	17/832	7/824
ST only strains			
	0 - 11	4/90	3/89
	12 - 23	3/218	4/214
	24 - 35	9/225	4/225
	36 - 47	6/180	4/176
	> 48	4/119	3/120
	TOTAL	26/832	15/824
LT only strains			
	0 - 11	8/90	3/89
	12 - 23	14/218	13/214
	24 - 35	13/225	7/225
	36 - 47	12/180	10/176
	> 48	7/119	7/120
	TOTAL	54/832	40/824
LT only and ST only strains			
	0 - 11	0/90	0/89
	12 - 23	0/218	0/214
	24 - 35	0/225	0/226
	36 - 47	3/180	1/176
	> 48	0/119	0/120
	TOTAL	3/832	1/824

Table 13. Seasonality of isolation of enterotoxigenic E. coli from passive surveillance of children with diarrheal illness cultured at the Santa Julia Consultorio or at the Calvo MacKenna Children's Hospital, 11/1/86 to 10/31/88

	Consultorio			Hospital		
Month	No. Cases Cultured	Total ETEC	%	No. Cases Cultured	Total ETEC	%
Surveillance Year 1, 11/86-10/87						
1986						
November	54 (0)	0	0.0	11 (3)	0	0.0
December	103 (94)	9	9.6	22(20)	0	0.0
1987 January	127(122)	18	14.8	18(13)	4	30.8
February	101 (92)	6	6.5	14(13)	1	7.7
March	117(105)	11	10.5	13(11)	2	18.2
April	77 (73)	3	4.1	10(10)	0	0.0
May	49 (46)	2	4.3	7 (7)	0	0.0
June	43 (38)	2	5.3	2 (1)	0	0.0
July	31 (26)	1	3.8	7 (6)	0	0.0
August*	26 (20)	0	0.0	4 (4)	0	0.0
September	35 (30)	0	0.0	3 (2)	0	0.0
October	31 (26)	0	0.0	9 (6)	0	0.0
Surveillance Year 2, 11/87-10/88						
November	51 (47)	1	2.1	14(12)	0	0.0
December	64 (59)	7	11.9	16(14)	1	7.1
1988						
January	76 (72)	9	12.5	18(14)	2	14.3
February	92 (92)	8	8.7	12 (9)	0	0.0
March	75 (71)	2	2.8	11(10)	1	10.0
April	35 (29)	1	3.4	11(10)	0	0.0
May	12 (11)	0	0.0	5 (5)	0	0.0
June	15 (13)	0	0.0	7 (7)	0	0.0
July	13 (12)	0	0.0	1 (1)	0	0.0
August	15 (13)	0	0.0	2 (2)	0	0.0
September	39 (28)	0	0.0	3 (3)	1	33.3
October	30 (24)	1	4.2	9 (6)	0	0.0
TOTAL	1311(1143)	81	7.1	229(189)	12	6.3

Table 14. Admission of newborn infants into a cohort to be prospectively studied up to 24 months of age for the occurrence of diarrheal illness and to identify illness-associated bacterial, viral and protozoal pathogens March, 1987 - October, 1988.

Month	Admissions	Monthly Cumulative Total	Ages (in months) of infants under surveillance: <1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
M	12	12	12&																			
1987		(0)	(0)+																			
A	12	24	12	12																		
		(3)	(1)	(2)																		
M	12	36	12	12	12																	
		(5)	(0)	(2)	(3)																	
J	12	47&	12	12	12	11																
		(2)	(0)	(1)	(1)	(0)																
J	1	59	12	12	12	12	11															
		(6)	(1)	(3)	(1)	(1)	(0)															
A	12	70	12	12	12	12	11	11														
		(4)	(0)	(2)	(0)	(1)	(0)	(1)														
S	12	82	12	12	12	12	12	11	11													
		(10)	(1)	(3)	(1)	(2)	(1)	(2)	(1)													
O	12	93	12	12	12	12	11	12	11	11												
		(9)	(0)	(0)	(1)	(2)	(1)	(1)	(3)	(1)												
N	12	103	12	12	12	11	12	11	12	11	10											
		(25)	(0)	(1)	(3)	(3)	(4)	(3)	(4)	(4)	(3)											
D	12	114	12	12	12	11	11	12	11	12	11	10										
		(27)	(0)	(1)	(0)	(2)	(3)	(5)	(3)	(7)	(3)	(3)										
J	12	123	12	12	12	12	11	10	10	11	12	11	10									
1988		(28)	(0)	(0)	(5)	(2)	(2)	(3)	(4)	(0)	(6)	(3)	(3)									
F	12	134	12	12	12	12	12	11	10	10	11	11	11	10								
		(24)	(0)	(2)	(1)	(6)	(1)	(2)	(2)	(0)	(1)	(1)	(3)	(4)								
M	0	134	0	12	12	12	12	12	10	10	10	11	11	11	10							
		(29)	(0)	(1)	(3)	(2)	(3)	(4)	(1)	(2)	(2)	(1)	(5)	(3)	(2)							
A	0	131	0	0	12	11	12	12	12	10	10	10	11	11	10	10						
		(38)	(0)	(0)	(2)	(2)	(6)	(2)	(2)	(0)	(3)	(2)	(6)	(2)	(1)	(3)						
M	0	123	0	0	0	12	11	12	11	12	10	10	9	11	11	10	10					
		(21)	(0)	(0)	(0)	(1)	(2)	(2)	(0)	(2)	(2)	(4)	(1)	(1)	(2)	(1)	(2)					
J	0	118	0	0	0	0	12	11	12	11	12	10	10	9	11	11	9	10				
		(22)	(0)	(0)	(0)	(0)	(3)	(1)	(0)	(0)	(0)	(2)	(5)	(3)	(0)	(1)	(3)	(4)				
J	0	125	0	0	0	0	0	12	11	12	11	12	9	9	9	10	11	9	10			
		(20)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(1)	(3)	(2)	(0)	(2)	(1)	(3)	(2)	(2)			
A	0	115	0	0	0	0	0	0	12	11	12	11	12	9	9	9	10	11	9	10		
		(26)	(0)	(0)	(0)	(0)	(0)	(0)	(2)	(4)	(1)	(3)	(1)	(4)	(2)	(1)	(2)	(2)	(3)	(1)		
S	0	123	0	0	0	0	0	0	0	11	10	12	11	12	9	9	9	10	11	9	10	
		(37)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(4)	(3)	(5)	(5)	(4)	(0)	(3)	(3)	(5)	(2)	(3)	(2)	
O	0	122	0	0	0	0	0	0	0	0	11	10	12	11	12	9	9	9	9	11	9	10
		(44)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(4)	(6)	(3)	(5)	(3)	(3)	(4)	(6)	(2)	(4)	(1)	(3)
Child mos. observ.			144	144	144	140	133	137	133	132	130	118	106	93	91	86	58	49	39	30	19	10
Diarrh. episodes			3	18	21	23	26	27	23	24	35	22	24	23	13	13	17	20	9	6	3	3
Diar./ch. mo. obs.			.02	.13	.15	.16	.19	.20	.17	.18	.27	.17	.22	.31	.16	.19	.29	.39	.23	.27	.16	.30
Total diarrhea days			20	146	225	182	260	227	158	211	242	154	259	211	71	78	100	103	56	82	14	13
Mean dur. diar. epi.			7.7	8.1	10.7	7.9	10.0	8.4	6.9	8.8	6.9	7.0	7.6	7.5	5.5	6.0	5.9	5.2	6.2	10.3	4.7	4.3
# epi. >14 days			0	2	4	2	3	2	2	3	3	2	3	1	0	1	1	0	1	3	0	0

No. of infants under surveillance

+ No. of episodes of diarrheal disease observed

& An occasional infant dropped out of the study.

Table 15. Preliminary data on etiologic agents identified in association with diarrheal episodes in the cohort of infants followed from birth to 24 months of age, March, 1987 through October, 1988.

	Cases								Controls						
	Age Group (mos.)							p	Age Group (mos.)						
Pathogen	0-3	4-6	7-9	10-12	13-15	16-18	19-21	value	0-3	4-6	7-9	10-12	13-15	16-18	19-21
Shigella flexneri	0	0	2	2	0	1	0		0	0	0	0	0	1	0
boydii	0	0	3	0	0	0	0		0	0	0	0	0	0	0
sonnei	0	0	0	0	0	0	0		0	0	0	0	0	0	0
all Shigella															
ETEC*	3	4	3	1	2	0	0	NS	3	2	1	1	0	0	0
EAF	5	6	7	3	1	0	0	0.012	2	1	3	2	1	0	0
EIEC	0	0	0	0	0	0	0		0	0	0	0	0	0	0
EHEC	0	0	0	0	0	0	0		0	1	0	0	0	0	0
Campylobacter	1	6	7	5	2	0	1	NS	2	5	4	3	2	0	0
Aeromonas	8	3	8	6	5	2	1	NS	3	3	10	9	7	1	0
non-typhi Salmon.	1	3	1	1	1	0	0	NS	0	2	0	0	0	1	0
Rotavirus	4	11	10	3	2	2	0	<0.000001	0	2	1	0	0	0	0
Yersinia	0	0	4	4	2	1	0	0.0063	0	0	0	1	0	0	0
Protozoa	1	1	5	9	9	8	0		1	1	3	3	10	2	1
Child mos. of observation:	572	408	380	280	175	88	10		572	408	380	280	175	88	10

* *E. coli* so far tested only with LT probe.

Note: LT, EAF, EHEC, EIEC probe data as of August, 1988.
Rotavirus results as of September, 1988.

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